7. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, measuring, and/or monitoring acrolein, its metabolites, and other biomarkers of exposure and effect to acrolein. The intent is not to provide an exhaustive list of analytical methods. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used for environmental samples are the methods approved by federal agencies and organizations such as EPA and the National Institute for Occupational Safety and Health (NIOSH). Other methods presented in this chapter are those that are approved by groups such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods are included that modify previously used methods to obtain lower detection limits and/or to improve accuracy and precision.

7.1 BIOLOGICAL MATERIALS

Data regarding the analytical methods used in the determination of acrolein in biological samples are limited. Boor and Ansari (1986) developed a method capable of detecting nanogram (ng) quantities of acrolein in biological samples. In this method, a derivatizing agent, 2,4-dinitrophenylhydrazine (DNP), is incubated with liver or kidney homogenate for a short period of time. The acrolein-DNP adduct is then extracted from the sample with chloroform. Analysis for acrolein is accomplished by elution on a reverse phase column using high performance liquid chromatography (HPLC), and detection of the adduct by ultraviolet (UV) absorbency. Interferences due to the coincidental elution of DNP adducts of ketones or aldehydes other than acrolein are not ruled out by this method of analysis.

In earlier attempts to quantify acrolein in biological media, Kissel et al. (1978) urged caution when using derivatization methods for the measurement of acrolein levels in biological media based upon data for analysis for acrolein in aqueous solutions (Kissel et al. 1978). Methods that utilized derivatives (DNP and 7-hydroxyquinoline) combined with colorimetric or fluorimetric detection were not specific for acrolein and consistently did not correlate with those obtained from bioassays. However, more recent studies have made better utilization of gas chromatography (GC) and HPLC techniques to improve the resolution of acrolein and acrolein derivatives from other interfering species (Al-Rawithi et al. 1993; Paci et al. 2000; Sakuragawa et al. 1999). These assays, in addition to other assays for quantifying acrolein in biological media, are summarized in Table 7-1.

Table 7-1. Analytical Methods for Determining Acrolein in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent Recovery	Reference
Urine	Urine is heated to 80 °C, head-space vapors sampled, and injected directly into GC	GC-MS	1–5 nM (0.056–0.28 μg/L)	7–87.9% (100 nM)	Sakura et al. 1998
Urine	Urine is reacted with derivatizing reagent (*m*-aminophenol/ hydroxylamine/ferrous sulfate) and heated to 100 °C for 15 minutes.	HPLC-FD[b]	<1 μg/L	99–104.1%	Al-Rawithi et al. 1993
Urine	Urine is centrifuged, lypohilized, and reconstituted in water. The acrolein metabolite, 3-hydroxyl-propyl-L-cysteine, is directly quantified.	HPLC-UV	1.25 μg/mL (1.25 mg/L)	No data	Sanduja et al. 1988
Urine	The acrolein metabolite, 3-hydroxypropylmercapturic acid, in urine is hydrolyzed to 3-hydroxylpropyl-L-cysteine in 2N HCl	Amino acid analyzer	No data	No data	Alarcon 1976
Plasma	Plasma is reacted with Luminarin 3[a] in 0.1 M sulfuric acid, extracted with methylene chloride, dried, reconstituted in acetonitrile	HPLC-FD[b]	5.6 ng/mL (5.6 μg/L)	78–82%	Paci et al. 2000
Tissue	Homogenized tissued mixed with 2,4-DNP stock solution, extract acrolein-DNP derivative with chloroform	HPLC-UV[b]	<0.2 ng (in extract re-constituted in 0.5 mL methanol)	4.6–43.8% (8 mg acrolein)	Boor and Ansari 1986

[a]Derivatizing agent
[b]Derivative analyzed

2,4-DNP = 2,4-dinitrophenylhydrazine; FD = fluorescence detection; GC = gas chromatography; HPLC = high performance liquid chromatography; MS = mass spectrometry; UV = ultraviolet

A method for the direct detection of acrolein in urine was developed by Sakura et al. (1998). In this method, a small volume of urine (0.5 mL) is heated to 80 °C, driving the dissolved acrolein into the headspace above the liquid. The headspace vapors are analyzed using GC-mass spectrometry (MS), which provides a detection sensitivity of between 1 and 5 nM (0.056 and 0.28 μg/L) acrolein in urine. The variability is due to differences in the ability to drive acrolein from various urine samples during the heating phase of the method.

Biomarkers of acrolein exposure have been studied based either on acrolein metabolites in urine or the formation of protein-acrolein adducts. Alarcon (1976) developed a method for quantifying 3-hydroxy-propylmercapturic acid (MCA), a known metabolite of acrolein, in urine. This method involves acidification of the urine to convert MCA to S-(3-hydroxypropyl)-L-cysteine. The amount of S-(3-hydroxypropyl)-L-cysteine can then be quantitated using an automated amino acid analyzer. Sanduja et al. (1989) directly measured S-(3-hydroxypropyl)-L-cysteine in urine at a sensitivity of 1.25 μg/mL using HPLC with UV detection (210 nm). Li et al. (2004) developed an enzyme-linked immunosorbent assay (ELISA) for detecting acrolein-protein adducts (APA) in albumin obtained from blood. The APA levels in plasma were found to increase by 32 and 58% in rates exposed to one or seven doses of 9.2 mg/kg/day acrolein, respectively. However, more work is necessary to correlate APA levels in plasma and acrolein exposures. Decreased activity of the enzyme, glucose-6-phosphate dehydrogenase (G6PD), has been found to occur when the purified form of the enzyme is exposed to 0.2–1.0 mM acrolein (Trieff et al. 1993). Studies have yet to be conducted to determine whether dose-dependent changes in G6DH activity are obtained *in vivo* as a function of exposures to acrolein.

7.2 ENVIRONMENTAL SAMPLES

The detection and quantification of acrolein in air is accomplished mainly through the formation of acrolein derivatives (Nishikawa and Sakai 1995). In the NIOSH method 2501 (NIOSH 1994), a known volume of air is pumped through a tube containing a support coated with the derivatizing agent 2-(hydroxymethyl)piperidine. The derivative is eluted from the tube with toluene, and analyzed by GC using a nitrogen specific detector (NSD). Variations of this procedure have also been reported. Rietz (1985) used DNP as a derivatizing agent on the adsorbent tube, and made a final analysis using HPLC coupled to a UV detector. Similarly, EPA method 8315A uses HPLC with UV detection to quantify the DNP derivative of acrolein that is obtained from the reaction of acrolein in air with 2,4-DNP coated on a silica matrix as air is pumped through a collection tube described in EPA method 100 (EPA 1996). The

DNP derivative is extracted with acetonitrile and analyzed using a C_{18} column and a detection wavelength of 360 nm.

Another method for quantifying acrolein in air involves the trapping of acrolein by bubbling air through an aliquot of ethanol, adding methoxylamine hydrochloride to form a derivative, and then brominating the resulting adduct to allow increased detector sensitivity. Quantitation is achieved by GC using an electron capture detector (ECD) (Nishikawa et al. 1986). A summary of these techniques, along with methods for other environmental samples, are presented in Table 7-2. Interferences due to coincidental elution of derivatives of the compounds can be a potential problem of these techniques depending on the resolving power of the chromatographic technique.

Derivatization methods for the measurement of acrolein levels in environmental media should be used with caution based upon data for analysis for acrolein in aqueous solutions (Kissel et al. 1978). In a comparison of chemical analytical methods to bioassays, results obtained using methods utilizing derivatives (DNP and 7-hydroxyquinoline) combined with colorimetric or fluorimetric detection were not specific for acrolein and consistently did not correlate with those obtained from bioassays. Certain direct methods of detection (nuclear magnetic resonance [NMR]), fluorescence, and differential pulse polarography) gave the best correlation to the bioassay results (see Table 7-2).

The analysis of acrolein in wastewater can be performed using EPA method 603 (EPA 2001c). In closely related techniques, an aliquot of water is subjected to a purge and trap protocol, and the sample is thermally desorbed onto a GC for analysis and quantitation. Coincidental elution of compounds with acrolein may lead to interferences in this method.

Ogawa and Fritz (1985) developed a method for the identification of acrolein in water. A known volume of water is passed over a column of zeolite that traps the acrolein. The column is then eluted with acetonitrile, and derivatization using DNP follows. By following this procedure, a sample that can be analyzed by HPLC is obtained. Similar approaches were used by Koostra and Herbold (1995) and Sakuragawa et al. (1999), but in their methods, DNP was coated on to a C_{18} matrix within a solid phase extraction (SPE) cartridge. The use of mass spectrometric techniques by Sakuragawa et al. (1999) to quantify acrolein-DNP derivatives provided for better detection sensitivities than the UV detection method used by Koostra and Herbold. Other derivatizing agents that have been used successfully for the

Table 7-2. Analytical Methods for Determining Acrolein in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air[a]	Collection on tube with 2-(hydroxylmethyl)piperidine[b] coated on XAD-2 resin, desorbed by toluene extraction	GC-NSD[c] (NIOSH 2501)	2 µg	No data	NIOSH 1994
Air	Collection on tube containing silica gel coated with 2,4-dinitro-phenylhydrazine, tube backflushed with acetonitrile	HPLC-UV (EPA 100/ 8315A)	0.03 ppb (500 L sample volume)	No data	EPA 1996
Water	Direct analysis using detector wavelength of 195 nm	HPLC-UV (EPA 8316)	30 µg/L	No data	EPA 1994a
Waste water[d]	Purge at 85 °C and trap onto methyl silicone/2,6-diphenylene oxide adsorbent, thermal desorption	GC-FID (EPA 603)	0.7 µg/L	104% (5 µg/L, RW); 80% (5 µg/L, POTW[e]); 2% (5 µg/L, IW[f]);	EPA 2001c
Waste water	Addition of isotopically-labeled standards, purge at 20–25 °C and trap onto methyl silicone/2,6-di-phenylene oxide adsorbent, thermal desorption	GC-MS (EPA 1624)	50 µg/L	No data	EPA 2001a
Air	Trap in ethanol solution, add methoxylaminehydrochloride[b], brominate	GC-ECD[c]	<4 ppb	81–96%	Nishikawa et al. 1986
	Trap on XAD-2 resin coated with 2-(hydroxymethyl)piperidine[b], desorb with toluene	GC-NSD[c]	NS	NS	Kennedy et al. 1984
	Trap on XAD-2 resin coated with 2,4-DNP[b], elute adduct with acetonitrile	HPLC-UV[c]	0.01 mg/m^3	No data	Rietz 1985
	Sampled air passed through ozone scrubber then through SPE cartridge where analytes are trapped on a C_{18} matrix coated with 2,4-DNP, elute adduct with acetontrile and dilute with water	HPLC-UV[c]	50–150 ng/m^3	>90%	Koostra and Herbold 1995
	Trap on C_{18} SPE coated with 2,4-DNP, extract with acetronitrile	LC-MS	0.44 ppb	100%	Sakuragawa et al. 1999

Table 7-2. Analytical Methods for Determining Acrolein in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water	Trap on Zeolite ZSM-5 column, elute with acetonitrile, derivatize with 2,4-DNP	HPLC-UV[c]	<10 µg	98%	Ogawa and Fritz 1985
	Nondirect measurement of aldehyde signal compared to signal for a calibrated sealed external TMS standard	NMR	5,000 ppm	NS	Kissel et al. 1978
	Dilution of sample with deionized water	Fluorescence spectrometer	>20 ppm	NS	Kissel et al. 1978
	Dilution of sample with deionized water, addition of phospate buffer and EDTA	Differential pulse polarography	>30 ppb	NS	Kissel et al. 1978
Personal Air	Trap on carbon coated with hydroquinone, desorb with 1,2-dichloroethane	GC	0.02 ppb	>75%	Hurley and Ketcham 1978
Rain	Add to collected sample methoxylaminehydrochloride[b], brominate	GC-ECD[c]	0.4 ng/mL	90–101%	Nishikawa et al. 1987b
Fats and natural oils	Fat or oil is emulsified in distilled water or 10 mM $HClO_4$, aqueous phase separated, then filtered and injected into column	HPLC-ED	0.15 µM	49–116%	Casella and Contursi 2004

[a]NIOSH method 2501 is the preferred method for quantitative analysis; method 2539 can be used to screen samples for acrolein.
[b]Derivatizing agent
[c]Derivative analyzed
[d]EPA method 603 is the preferred method for quantitative analysis; method 624 can be used to screen samples for acrolein.
[e]POTW = prechlorination secondary effluent from a municipal sewage treatment plant
[f]IW = industrial waste water containing an unidentified acrolein reactant

2,4-DNP = 2,4-dinitrophenylhydrazine; ECD = electron capture detector; ED = electrochemical detector; EDTA = ethylenediaminetetraacetic acid; FID = flame ionization detector; GC = gas chromatography; HPLC = high performance liquid chromatography; LC = liquid chromatography; MS = mass spectrometry; NMR = nuclear magnetic resonance; NS = not specified; NSD = nitrogen specific detector; POTW = publicly owned treatment works; RW = reagent water; SPE = solid phase extraction; TMS = tetramethylsilane; UV = ultraviolet

monitoring of acrolein in the environment include dimedon, phenylhydrazone, 4-hexylresorcinol, and 3-methyl-2-benzothiazolone (Altshuller and McPherson 1963; Peltonen et al. 1984).

Direct detection of acrolein in water is obtained using EPA method 8316 (EPA 1994a). The water sample is injected on to a C_{18} HPLC column and then quantified by UV at a wavelength of 195 nm.

7.3 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of acrolein is available. Where adequate information is not available, ATSDR, in conjunction with NTP, is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of acrolein.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

7.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect.

Exposure. Methods have been identified that measure the concentrations of acrolein in blood, tissues, and urine (Al-Rawithi et al. 1993; Boor and Ansari 1986; Paci et al. 2000; Sakura et al. 1998). The methods used for the analysis of acrolein, however, can be susceptible to interferences.

Several studies have been identified that explore the use of specific biomarkers that can be associated quantitatively with exposure of acrolein. There are methods that can detect 3-hydroxypropylmercapturic acid, which is a metabolite of acrolein, in urine (Alarcon 1976; Sanduja et al. 1989). Changes in the activity of the enzyme, glucose-6-phosphate dehydrogenase, and levels in acrolein adducts of albumin in blood as a function of exposure to acrolein have been studied (Li et al. 2004; Trieff et al. 1993), but further work is necessary to correlate exposures of acrolein with changes in the biomarkers *in vivo*.

Effect. There are no biomarkers that have been associated quantitatively with an acrolein-induced effect in humans. Identification and use of such biomarkers would be useful for establishing a more reliable assessment of the relationship between acrolein intake and acrolein-induced effects in humans than would be provided from monitoring data of acrolein concentrations in air, drinking water, or food.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Suitable methods are available for the determination of acrolein in air, which mainly involve the formation of acrolein derivatives (Altshuller and McPherson 1963; Nishikawa and Sakai 1995; Peltonen et al. 1984) that are quantified using GC (Kennedy et al. 1984; NIOSH 1994; Nishikawa et al. 1986) or HPLC techniques (EPA 1996; Koostra and Herbold 1995; Rietz 1985). Suitable methods are also available for analyzing acrolein in water that either directly quantify acrolein in water (EPA 1994a; Kissel et al. 1978) or use an extraction step followed by either analysis of acrolein (EPA 1992) or acrolein derivatives (EPA 2001c; Ogawa and Fritz 1985). Nevertheless, new methodologies for the determination of acrolein have been reported. An LC-MS based method (Sakuragawa et al. 1999) can offer increased sensitivity and greater ease of performance than is currently available for the direct analysis of acrolein in water (Rietz 1985). Standardized methods for analyzing acrolein in soil were not located. However, given the extent to which acrolein is expected to volatilize from soil based on its high vapor pressure (274 mm Hg at 25 °C) and the irreversible binding of acrolein in soil, the lifetime of acrolein in soil may be too short for concern from the standpoint of being a source of human exposure to acrolein. Therefore, development of analytical methods for measuring acrolein in soil is not expected to provide data that would be useful in assessing human exposures to acrolein.

7.3.2 Ongoing Studies

The EPA is conducting a study on human exposure to air toxics, which includes optimizing the PAKS method for measuring airborne acrolein. At the University of California at Davis, Dr. M.J. Charles is developing an air sampling method that uses a Cofer scrubber for measuring acrolein in ambient air. Also at the University of California at Davis, Dr. Shibamoto is measuring the concentrations of acrolein in a variety of cooked foods and in beverages. Dr. Iype at the Biological Research Facility in Ijamsville, Maryland, is developing an antibody-mediated detection system for assessing human exposure to acrolein that is based on the quantification of acrolein-DNA adducts in white blood cells. No other ongoing studies concerning the determination of acrolein in environmental media or biological materials were identified.